Metamorph/Nicon Microscope Instructions

No Food or Drink Permitted in the Microscope Room
CRITICAL: The microscope can be seriously damaged by improper care of lenses. So review the following very carefully before turning on the microscope.

Lens care: Jarring a lens in any way can permanently destroy the alignment of the elements within it. Handle lenses very gingerly. Before examining a sample, make sure there is no foreign material on the sample that could contaminate the lens surface. After use, clean lenses while in the nosepiece and only with lens paper. Fold the paper several times and rub it back and forth over the lens surface. Use several pieces of lens paper until there is no longer evidence of oil on the paper. Avoid pressing the lens paper with your finger into the transparent part of the lens. If the lens becomes contaminated with any substance, contact the facilities manager for instructions on cleaning. Don't even think of applying any solvents to the lens surface.

The lenses for this microscope are 20X (water, glycerol, or immersion oil, three positions on the correction collar for different immersion media), 63X and 100X (immersion oil phase-contrast objectives), which should be used with PH3 phase-contrast ring.

What are the advantages of this imaging system?

Main advantages of this system are the ability to image live cells and the ability to quantify the images. This imaging system consists of fluorescence wide-field inverted microscope, sensitive 12-14 bit camera and Metamorph software. Xenon light source is benign for the live cells. Digital Orca II camera allows rapid acquisition, which is important for imaging of live specimens. This system is applicable for any multi channel imaging, time lapse imaging and z-scanning through the cells. Sophisticated Metamorph system is best suited for quantification. Images and stacks of images may be modified in Metamorph, converted to movies and exported to Adobe Photoshop, Adobe Premiere. Results of quantification may be exported to Excel.

Filters

The system is equipped with automatic filterwheels. One may automatically collect images in three channels: BFP (DAPI), GFP (FITC), DsRed (TRITC). The system also is equipped with filter cubes for BFP (DAPI), GFP (FITC), DsRed (TRITC) and CFP.

Turning on the system.

1.) Turn on the xenon lamp power supply (fluorescent light). Due to high voltage this unit should be switched on first and switched off the last, otherwise the other parts of the system will be damaged.
2.) Turn on the halogen lamp (transmitted light).
3.) Turn on the shutter and filterwheel controller (Lambda 10-2), camera controller (Hamamatsu) and z-step motor controller (Prior), which are on the shelf above the monitor.
4.) Turn on the computer and its monitor. Start the Metamorph program.

Viewing the slides.

Switching between viewing and imaging. On the right side of the stand there is a dial operating the beam splitter. Different positions of the beam splitter are explained on the panel on the front side of the stand. We use either A position (all light sent to the eyepieces) or C position (all light sent to the camera).

Putting appropriate objective in place. The objectives are changed manually. The lenses for this microscope are 20X (water, glycerol, or immersion oil, three positions on the correction collar for different immersion media), 63X and 100X (immersion oil phase-contrast objectives), which should be used with PH3 phase-contrast ring.

Focusing. The coarse and the fine focus knobs are the large ones on both sides of the microscope stand. Normally, the z-step motor is disengaged, and you should experience no problem in focusing. However, the right focus knob may be attached to z-step motor. If this motor is engaged, the fine focus moves in steps rather than continuously, and one may experience difficulties in focusing. Focus with a

black knob on the side of the gray joystick box to the right side of the scope. Please, ask staff to disengage the step motor for better focusing, if you are not going to use z-step motor, i.e. you are not planning to scan through z-axis of the cell.

Putting the slide on the stage. If you are using immersion oil, apply a small drop and put the slide on the stage. Do not mix immersion oils. If your slide has oil from another bottle, clean it first. If there is any other contaminant on your slide, clean it, or do not use that slide. Contaminating the lens surface is a very serious problem. Place your sample on the stage and then carefully raise the objective using first coarse, then fine focus knob. Raise the stage until the objective just flattens out the immersion solution drop as the objective contacts your slide. Check your slide periodically to make sure you are not over-focusing and pushing up the objective with the slide. This can seriously damage the lens.

Transmitted light:

The switch for transmitted light is located on the left side of the microscope stand. To view the specimen in transmitted light switch it on. The small black knob next to the large focus knob allows you to adjust the brightness of transmitted light. Do not forget to put PH3 phase-contrast ring in place, if you are using phase-contrast objectives. Position A on the phase-contrast ring changer corresponds to bright field illumination. The field diaphragm for transmitted light is located on the very top of the stand, above the condenser, and it is labeled by F mark. The aperture diaphragm is located just above phase contrast ring of the condenser. For transmitted light daylight filter (NCB) should be in all the time. Green filter (GIF) should be in while doing phase-contrast. ND slider is empty.

Fluorescence:

Info about filter cubes and filter wheels: For focusing on your specimen, use filter cubes, which are changed manually. The filter cubes are in the slider below the nosepiece. The filters are from left to right BFP (DAPI), GFP (FITC), DsRed (TRITC). The last position is occupied by triple dichroic mirror that should be put in place if filters in filter wheels are to be used. CFP and YFP filter cubes are in a separate slider. Ask staff to show you how to change a slider. **Please, always put back a slider with 4 main filter cubes after you finished your session.**

If you need to collect images in single channel, you may continue using filter cubes in the slider. If you want to acquire images in several channels, it is better to use automatic filter wheels. One may automatically collect images in three channels: BFP (DAPI), GFP (FITC), DsRed (TRITC). To collect images automatically, first put the triple dichroic mirror in place. The triple dichroic mirror is in the far right position of the filter cube slider under the nosepiece. You may use automatic filter wheel if you want to image in single channel, but then do not forget to put the triple dichroic mirror into the light path.

Further down you will read about the commands on the taskbar that allow you to collect images using filter wheels. Remember, that if you are using just filter cubes, you cannot use those commands, and you should ensure that you put **filter wheels in open position**, so that there will be no extra glass in the light path.

Opening the shutter. Switch off the transmitted light with the switch at the bottom left side of the microscope stand. Put the appropriate filter cube into the light path.

To view the specimen in fluorescent light you need the Metamorph program running. On the "basic tools" toolbar click on ‘Open fluorescent shutter'. If you see no light, check first whether the manual shutter slider is opened. This shutter is operated by a slider on the left, the nearest to the user. The slider should be in to open the shutter. If the shutter is open and still you get no light coming through, click on ‘Open Both Filter Wheels”.

While focusing, open the shutter with ‘Open fluorescent shutter' command, and then use the mechanical shutter.

Optimizing the brightness of fluorescence. Several devices may affect the brightness of your specimen. The next two sliders after the mechanical shutter slider contain fluorescence neutral density filters. ND filter sliders should be out if the filters are in.

There is a field stop in the fluorescent light path. Reduction of the field stop reduces out-of-focus light. If you are uncertain about the field stop, please, ask staff.

Collecting data.

The beamsplitter on the right bottom side of the microscope stand should be in position ‘C’ – all the light goes to the camera.

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Further down you will read about the commands on the taskbar that allow you to collect images using filter wheels. Remember, that if you are using just filter cubes, you cannot use those commands, and you should ensure that you put filter wheels in open position, so that there will be no extra glass in the light path.

Adjusting the settings.

First, adjust the settings for imaging. Open "Acquire/Acquire from Digital Camera" dialog window. Select appropriate routine: DAPI, GFP, etc, from predefined Acquisition Settings. Browse among the Acquisition Settings, clicking on black triangles. Click on "Define acquisition settings" button. Select the region of interest. We recommend "Full Chip". You may choose the "automatic scaling" to view the dim features. You may also choose between fast or slow acquisition (larger dynamic range). Select the exposure time.

If you are going to use automated filter wheel, please, put the double dichroic mirror into the light path. Click on "Open DAPI (or FITC, or TRITC) channel" button on a basic Toolbar. If you are going to use the filter cube, this step is not necessary.

Click on "Expose and Transfer" in the Acquire from Digital Camera" dialog. Examine the image. Check the grayscale values of the image. You may point to different pixels of the image and read the values on the bottom bar of the window. Enhance the exposure time if necessary. You may also let the program to find exposure automatically, but we do not recommend it, because the specimen will be bleached in process. You may also adjust binning and gain. The system will use the last settings that you input for the appropriate channel in the "Acquire from Digital" dialog.

Collecting images and image series.

After you have adjusted the settings for each channel, you may collect single images, or you may collect images in several channels plus overlay. Use the commands from the "Basic Tools" menu. For single images use "Acquire DAPI image", etc. For double and triple images and color overlays you may choose "Acquire DAPI FITC and Overlay". Remember, that triple dichroic mirror should be in place to use the filter wheels. **Those commands will not work with filter cubes, only with filter wheels.**

Time lapse imaging, and z-series.

In addition to collecting a series of images in multiple channels you may also do Z-scans (collect multiple focal planes along the z-axis of the cell) and do time lapse imaging. For z-scans please, engage the z-step motor.

Saving and Storing Data

Your data should be saved first as 16 bit tiff files to ensure that they will retain all the necessary information. They also may be scaled to 8-bit images that can be opened by Adobe Photoshop. If your images are color encoded, or you created overlays, save them as 24 bit color images. By pressing ALT/I when the file is opened you may get all the necessary info about how the file was collected. **Write down the objective data - they are not saved in the file info.**

While acquiring images save your data in your folder in D:\Your directory. **Unfortunately, the default is disk C, so make sure to save on disk D, otherwise the program will not run properly**. For each session create subfolder with the name specifying microscope and date (for example, MN071099).

After session you need to remove your data from the computer Data kept on Metamorph computer for more than a week will be erased. You may FTP your data to Terabyte Server (see below). Use the program WS_FTP95, the icon for which can be found on a desktop. Also, you may write your data to the CD, but only within the time frame you signed up for. Please, bring your own CD if you plan to do so.

Shutting down the system (If the system will be used within two hours, then only wipe oil from the lenses. Leave everything else on. Sign the logbook. Turn off the room lights).

1) Move your data to the SC-B41-SSA_SSA_41_G1_SERVER/SSA_G1 /LRBGEIMAGE/YourFolder. This server will be automatically mounted on all the computers of the facility. If you will not see this server mapped on your personal computer, map it yourself. Find red N in the lower right corner of the computer screen, right-click on it. Select Novell Map Network Drive and for the "network path to resource" field, use the following syntax \\lrbgeimage.nci.nih.gov\ssa_41_g1\lrbgeimage. You may also write your data on a CD or Zip disk.
2) Exit the program.
3) Switch off the three controllers
4) Put the objective in the lowest position. Wipe off the oil from the objective. Switch off the halogen and xenon lamps.
5) Cover the microscope with plastic cover.
6) Sign the logbook and note any problems.
7) Turn off the room lights, and check once more if any microscope-associated lights remain on.